ISTITUTO SIEROTERAPICO MILANESE
"SERAFINO BELFANTI"

Ente Morale Aggregato alla Università di Milano

Via Darwin, 20 - MILANO
TEL. 30.640 - 30.677 - 33.787 - 32.823 - 33.917

10/7/53

Dear Joshua,

Your last letter just received. Meanwhile proofs have come of our joint paper; I have accepted all your corrections (except a few minor ones, and these mainly for typographical reasons; they are marked NO on the manuscript). I am therefore sending back your pages, so that you can reconstitute the full manuscript; I had always forgotten to send the second copy. Re the statistical issue : I have dropped the relevant paragraph as of little importance. I do not think I was wrong, though I certainly overrated the importance of the hypothetical model leading to extra Poissonian variance. Just to explain me better than before with an extreme example: suppose you seed 10^8 cells, each from a parallel culture, on drug plates; and each culture contains 10^{-7}, i.e. 10 cells on; average, of some peculiar type, say snakes or some other odd type of cells. Actually, their number will vary from culture to culture even in the most strictly identical conditions, according to a Poisson ; there may be cultures which have 5 snakes, others which have 20 etc. Suppose, moreover, that the probability of a normal cell getting resistant is 0, while snakes have a probability of 10% of getting resistant by some lamarckian mechanism. There will be on average 1 resistant "mutant" per culture, but their number will vary from culture to culture with a variance higher than a Poisson, like a Poisson superimposed on another (Neyman's contagious distribution , I think ; no exact quotation at hand). The scheme could be easily extended, for instance to a continuous variation of the probability of giving resistance for different types of odd (rare) forms. It is essential that the odd type of cells, which can become resistant, are rare; otherwise their number per culture will show little variation from culture to culture.

I am enclosing cultures of W 583-not checked here in the last two or three years, I am afraid, but it could not improved upon if found unsatisfactory; I hope it will meet your needs. Also enclosing strain 8 ($M \rightarrow F^r N y^r$) and an F^r recombinant from it x W 945. The latter can be infected but with considerable difficulty; it still segregates $F^+ : F^-$ in the progeny. These strains were promised months ago, and not sent, Sorry. They stayed on my table and got lost among sheets.

Thank you very much for your generous offer concerning my stay at Madison. I shall see what I shall be able to do about improving on dates of my coming. I have written to the Rockefeller, who answered in an uncompromising way.++ WHO is a good idea, but I have no good strings to pull there. Fulbright is another chance, for the journey only.

++. Pomerat says he will probably come to Europe next autumn and may be able to get some decision at that time. Too many applicants probably.

I had a hope of restarting some cytology, by taking movies of an Hfr plate at the microscope. This project has to be stopped until Octo-

ber. I hope we shall be able to collaborate on the cytology before my coming there. I have considerable objections to looking myself through a microscope because I get easily bored by this, but the idea that I might have a camera looking for me has revived my interest for it.

Jinks' coming allowed me to restart on the work of formal genetics of K-12. In the last five or six weeks it has been possible to work at full rate although I had very little time to spend in the lab. I have often opportunities for good technical help, and this time it could be exploited completely because of Jinks' constant supervision. Some ten thousand prototrophs or more were thus scored and the work is not yet finished. The programme which I was thus able to set to work was the following : obtain, from Hfr x TLB_1-sugar marked crosses, F- progeny of all combinations for Met, Lac, Az, Ara (linked with TL) and Gal (linked to Lac, left of it). Make crosses in all possible combinations, selecting always from $M\text{-}S^r$ x $M\text{+}S^s$ on minimal streptomycin, after infection with F+ of either or both parents.

An important by-product of this research was the following. When Gal+ recombinants from HfrGal+ x W 945 (Gal-F-) were selected for (they are very rare, certainly below one% and possibly much rarer), all these recombinants proved to be Hfr, except a few, no matter what they were for other markers ! Thus, Hfr can segregate regularly, more or less like any other locus; but it is usually eliminated, and so are the markers linked with it, elimination being less drastic, for markers more distant from it, like T_6 or Dat.

This prompted me to advance the following theory. A single point (it may be a short region, but more of this later) of the F+ chromosome is eliminated; it is very near Hfr or Hfr itself, it may be called the "centromere" (without any really serious reason at this time). If this is true, irrespective of whether an F+ or an Hfr is crossed to an F-, the following consequences would be drawn :

1) our crosses lead to a map (with a single chromosome): M-St-Gal-Lac-Az-Ara. There are difficulties of pairing especially at Gal, less in the rest (see also Rothfels data, TL-supplemented cross); pairing varies significantly from line to line.

2) The standard cross M-F+ x TLB_1-F- is consistent with this theory. The point left of Lac, mapped as M, is actually the centromere, which must always be that of the F- parent. The best support of the theory comes from Newcombe and Nykolme's data. Map as follows : M-Xyl-Mal-S-Gal-Ara-Cen-Lac-T_1. Only double and quadruple crossovers between M and Cen will be viable. No quadruple is found; doubles show some slight negative interference (incomplete pairing? this seems unavoidable on <u>any</u> theory). Similar results from your cross A, table 5, CSHS 1951; cross B is F+ x F+ ; also from my cross 1950, Boll. IS.M.

3) Data from our JGM paper consistent with the theory, assuming map B_1(M)--Xyl-Mal-S-Cen-Gal-Lac-L(Ara)T. In crosses TLB_1-S^rF+ x TLB_1+F-, double c.o. necessary in region right of Cen for recombination of Lac or Gal; therefore very rare (rarity accentuated by pairing troubles).

More data came from F+xF+ crosses in the $M\text{-}S^r$ programme. Here, both F+xF+ and Hfr x F+ are better than F+xF- or Hfr x F- crosses; yields are as high or higher than F+xF- or HfrxF- crosses, and elimination or pairing troubles seem smaller. This is, I think, the best point now available for postzygotic elimination. Since an HfrxF+ or F+xF+ cross works with less elimina-

ISTITUTO SIEROTERAPICO MILANESE
"SERAFINO BELFANTI"

Ente Morale Aggregato alla Università di Milano

Via Darwin, 20 - MILANO
TEL. 30.640 - 30.677 - 31.757 - 32.823 - 33.917

tion than when/the parent is F-, the conclusion seems unescapable that elimination must be postzygotic and/must be the consequences of the interaction between the two parental centromeres, unless one assumes that the F- parent evokes the formation of gametes from the F+ strain, which have lost segments, while the F+ do not. Possibly, infection of the F-(centromere, if one assumes F and Hfr occupy the same locus) and elimination of the F+ centromere(and linked markers which have not crossed over) are strictly correlated. These conclusions may be affected(or be made easier)by the fact that there is here selection for the S-Mal region, usually eliminated.

Two points need consideration now. Is Gal of W 945, which is linked with Hfr, the same as Gal_4? I have no Gal_4 in my collection. From Rita, Rome, I had a $lambda^s$ Gal_4-strain, W1294; linkage between this Gal (but is it a Gal_4) and my Gal, as well as between Hfr and lambda being tested now. If, as possible, lambda, and Hfr(the possible seat of F+) turn out to be closely linked, we have a region showing a high elimination and high concentration of attached viruses, a rather unique fact which may have some deep significance. Lambda may be able to transduce Hfr in this case. Perhaps you could tell me whether Gal of W 945 is allelic to Gal_4, and airmail me a safe Gal_4 -$Lambda^s$(better if F-).

The second point is: how is this connected with the data from diploids? I have been unable to find an answer. Perhaps in many cases, at least those in which diploids arise, elimination extends to S-Mal, the regions nearest to the eliminated centromere. The F+ centromere is destroyed and destruction extends to neighboring regions, though not regularly; the loose chromosome end(broken between say Xyl and Mal)attaches itself to the (previously) F- centromere. But this may be day-dreaming. Another possibility is that the S-Mal region is actually terminal, as in your 1951 map, and is easily lost; the centromere is lost too, and this creates spurious linkage.

Our $M\text{-}S^r$ programme had been devised as a means of selecting on one chromosome, and checking what happens in the other, with all cares given to viability tests etc. We found it easier to explain the data by a single chromosome and assume that while selected markers were on one arm, free markers were all on the other (the right arm). We now try to develop strains which can be selected on the right arm, allowing the left arm to segregate. This should help considerably to solve the problem. In any case, pairing seems to be defective in all instances tested. This creates apparent negative interference and makes the tests for linearity ineffective.

In view of the discrepancy between your conclusions(with Fried), and ours, I should very much like to know with more detail what kind of experiments led you to postulate two chromosomes; they might be the same as our two arms. A variety of schemes might explain why two cytological chro-

might segregate like a two-armed one, in some conditions, and not in other ones.

The possibility that chromosome aberrations increase difficulties is not entirely excluded. Some differences between lines were noticed in our crosses. On the other hand, the data of the JGM paper should be homozygous for chromosome aberrations.

Before Jinks leaves, end of this month, we shall attempt to summarise our data, and shall let you have the result of our joint effort. I shall communicate these data to the Genetics Congress, as they form a better work than that previously done this year (F^r data and F+, kinetics; incidentally, F^r data do not fit satisfactorily with the rest of the theory, but since F^r originals seems to contain some possible chromosomal trouble, which might explain the reduced frequency of recombination, it deserves further study which I shall start as soon as less urgent work will be over; and it may be only apparently at variance with the rest).

Having done nothing for the Microbiology Congress, for which we had planned a joint paper, I feel rather embarrassed now that only few days are left for filling the gap. I shall send an uncompromising summary, like the enclosed one, with the proviso that if you do not feel like joining in such a paper, we can disjoin in time. If you have a manuscript of any length which you want me to read at the Microbiology Congress for you, in lieu of the above joint paper, or in any other way please let me know (by return of mail).(++)

The filter arrived two weeks ago in good conditions. It has now been welded and we shall soon test it. Thank you very much.

Coli B: two independent strains of coli B, of different origin, gave same result. Progeny usually seems F-, but Calef has too few markers available; previous results showing Hfr progeny are doubtful. In some coli B x K12 crosses unstable heterozygotes seem to form. All these intervarietal crosses give, somewhat expectedly, complicated results.

(++) I cannot foresee now when I shall be able to write the paper in extenso. Perhaps just a day before.

Busily yours

Luca.